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The interaction of HSV-1 tegument proteins pUL36 and pUL37: a novel target for antivirals that inhibit viral assembly



“Given that interacting homologues of pUL36 and pUL37 are present across the *Herpesviridae* family ... the opportunity exists for developing a generic antiviral drug that targets their interaction.”

Russell J Diefenbach^{*1}, Cornel Fraefel² & Anthony L Cunningham¹

Infection with human pathogens herpes simplex virus type 1 (HSV-1) or the closely related HSV-2 is for life. The herpes simplex viruses infect orogenital stratified squamous epithelium, then enter cutaneous nerves and are transported to the dorsal root ganglion where they remain latent for life. Periodic reactivation and transport back along the same nerves leads to recurrent disease close to the site of initial infection or asymptomatic shedding of virus in secretions. Infection causes ocular disease or encephalitis in immunocompromised adults and severe disseminated infection and encephalitis with high mortality and neurological impairment in neonates. Over one third of the world's population suffer from recurrent HSV infections several times a year and are thus capable of transmitting HSV by close personal contact.

Some of the most effective and non-toxic antiviral drugs currently available,

acyclovir (Zovirax), valaciclovir (Valtrex) and famciclovir, are used for treating herpes simplex and varicella zoster virus infections. They have been modelled upon the discovery of nucleosides with antiviral activity isolated from sea sponges such as *Cryptotethya crypta*. These selective DNA polymerase inhibitors belong to the class of nucleoside analogues, but instead of ribose they contain an open polyol structure. The mechanism of action involves progressive phosphorylation by viral then cellular thymidine kinases. The end active metabolite, acycloguanosine triphosphate, has approximately 100-times higher affinity towards viral polymerase than the cellular enzyme. Thus, it is preferentially incorporated into the viral DNA and because it lacks a 3' end no more nucleotides can be added, resulting in premature chain termination [1].

Extensive clinical use of these drugs has led to the emergence of resistant viral strains.

KEYWORDS

• antiviral • herpes simplex virus
• pUL36 • pUL37 • tegument • virus assembly

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While resistance is not a serious issue for immunocompetent individuals, it is a serious issue for immunocompromised patients, especially those with HIV or for those who have undergone organ transplantation. Furthermore, these drugs reduce the frequency and magnitude of viral shedding of HSV-2 from the anogenital tract of infected women but frequent breakthrough episodes occur, probably due to their short half-life [2]. This is important, as there is strong evidence that prior infection with HSV-2 increases HIV acquisition, and reactivation of HSV-2 increases HIV replication and transmission [3]. Attempted suppression of genital HSV-2 shedding with these drugs has not prevented HIV acquisition [4]. Given that no effective vaccine is currently available for HSV, there is a crucial need for the development of new anti-herpes drugs with a longer half-life that can inhibit mucosal infection and shedding by both wild-type viruses and drug-resistant strains, especially to prevent asymptomatic transmission and to reduce HIV acquisition and transmission [3]. Clearly, novel nontoxic drugs that are more efficacious and target different viral functions than those presently available would be of great use, especially for resistant viruses in immunocompromised patients. In particular, the targeting of different stages of herpesviral replication would allow combination with current drugs (which all have the same site of action) and aid in more rapid resolution of disease and minimise antiviral resistance [1].

In molecular herpesvirus research there has been a shift away from the well characterized viral capsid proteins and glycoproteins towards the less well-characterized viral tegument (bridges capsid and glycoprotein envelope) and its role in viral replication [5]. A number of studies, including our own, have now identified the interaction of tegument proteins pUL36 and pUL37 and their homologues across the *Herpesviridae* family [6–10]. The interaction of pUL36 with capsid proteins provides the platform for subsequent recruitment of remaining tegument, including pUL37 [11].

For HSV-1 and the related swine pathogen Pseudorabies virus (PrV), deletion of the genes encoding either of pUL36 or pUL37 blocks secondary envelopment in cell lines (process reviewed in [12]) of capsid and subsequent virion maturation [11,13–17]. In the case of HSV-1, residue D631 in the C-terminal pUL36 binding domain of pUL37 is essential for binding pUL36 [18]. Deletion of the 25 amino acid pUL37-binding domain, located

in the N-terminus of HSV-1 pUL36 [19], revealed that the interaction of pUL36 and pUL37 is essential for the process of secondary envelopment during virion assembly [20]. The interaction of pUL36 and pUL37, which forms the inner tegument layer adjacent to the viral capsid, appears to be important for both trafficking to the site of secondary envelopment [11,17,20], as well as subsequent recruitment of remaining viral components through direct interactions with either tegument or envelope proteins [21,22].

Recently, the structure of the N-terminal half of PrV pUL37 has been elucidated and appears to resemble components of multi-subunit tethering complexes that regulate vesicle trafficking in cells [23]. This is consistent with a role in trafficking during viral assembly, but whether this occurs in conjunction with binding of pUL36, via the C-terminal half of pUL37, and/or other host cell proteins remains to be determined. The structure of full-length pUL37 or the C-terminal half of pUL37, which contains the pUL36 binding domain [18,24], has not yet been reported. It may well be that the presence of bound pUL36 results in conformational changes in pUL37, which enhances stability of the two proteins. Preliminary data from our laboratory does suggest that coexpression in bacteria of a minimal fragment of pUL36, containing the pUL37 binding domain, and the C-terminal half of pUL37 results in enhanced expression levels of pUL37. Certainly, there is increasing evidence that pUL36 is also stabilized by bound pUL37, which may prevent proteasomal degradation of pUL36 during the course of infection [18,20].

The growing body of knowledge on the nature and essential role of the interaction of pUL36 and pUL37 in HSV assembly now provides a strong basis for developing therapeutics that block the assembly of HSV. Successful soluble expression of pUL36 and pUL37 interacting domains, and subsequent knowledge of the structure of a complex of pUL36 and pUL37 would be a significant step in aiding the discovery of small molecule inhibitors that block viral assembly. This would represent a different strategy to the current inhibitors of HSV DNA replication, which include those that either directly or indirectly target viral DNA polymerase and those in clinical trials that inhibit viral helicase-primase [1]. It would also allow combination therapy with currently available antiviral drugs (all of which have the same mode of action) aimed at different stages of the HSV replication cycle, similar to

“...the targeting of different stages of herpesviral replication would allow combination with current drugs ... and aid in more rapid resolution of disease and minimise antiviral resistance...”

that for HIV and hepatitis B virus. In addition to targeting assembly, the development of inhibitors of HSV entry also provides much needed diversity in drug design [25].

Given that interacting homologues of pUL36 and pUL37 are present across the *Herpesviridae* family (reviewed in [5]), the opportunity exists for developing a generic antiviral drug that targets their interaction. Although the overall amino acid homology between homologues is relatively small, the probability of structural similarity for interacting domains is likely to be high, given they have similar roles in viral assembly. Due to the conservation of the interaction of pUL36 with pUL37, knowledge gained with HSV and PrV

will also facilitate our understanding of assembly across the *Herpesviridae* family. Furthermore, it will provide essential information for development of attenuated HSV vaccine candidates.

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